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Viral load of human papillomavirus and risk of CIN3 or cervical cancer

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Carcinogenic human papillomaviruses (HPV) are thought to be necessary for development of cervical cancer. We assessed whether higher viral loads of such viruses predicted future risk of CIN3 or cancer (CIN3+) in a cohort of 20 810 women followed up for 10 years with cytological screening. We measured the viral load for 13 types of carcinogenic HPV (relative light units normalised to 1 pg/mL HPV 16 positive controls [RLU/PC]) using Hybrid Capture 2 testing of cervicovaginal lavages obtained at enrolment. Results were stratified into four groups (RLU/PC 1 to <10, 10 to <100, 100 to <1000, ⩾1000). Although presence of HPV strongly increased risk of CIN3+, high viral load did not further predict risk of CIN3+.

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Carcinogenic human papillomaviruses (HPV) are regarded as necessary causal agents of cervical cancer. Cervical specimens of women with cervical lesions (malignant or premalignant) have higher average viral loads than women who are positive for HPV but are cytologically negative. The relation between viral load and future risk of cervical intraepithelial neoplasia 3 or cancer (CIN3+) is less certain.

Investigators of a case-control study[1] reported that the viral load of HPV 16 in archival Pap smears was a risk factor for development of carcinoma in situ (equivalent to CIN3), such that higher loads of HPV 16 DNA in the cervix, up to 13 years before diagnosis of carcinoma in situ, strongly predicted development of disease. In our Portland Kaiser Permanente cohort study,[2] we examined the association of HPV viral load with future CIN3+.

At the enrolment visit, patients (>95% participation) had routine Pap smears. Their cervix was then rinsed with 10 mL sterile saline and the pooled fluid obtained from the posterior vagina. We tested frozen aliquots (–70°C) of the lavages by Hybrid Capture 2 (Digene Corporation, Gaithersburg, MD, USA) for a group of 13 carcinogenic types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Signal strengths in relative light units were compared with 1 pg/mL HPV type-16 DNA positive controls (RLU/PC), and specimens with ratios of one or greater RLU/PC were judged positive.

The cohort was established in 1989–90 and included an unbiased sample of about 50% of women undergoing cervical cytological screening at Kaiser Permanente, OR, USA. Women were followed up to 122 months, undergoing a median of three repeat smears (range 0–30), with 83% of women having at least one repeat smear. Age at enrolment ranged from 16 to 94 years (mean 35·9, SD 12·6). Women with negative smears at enrolment had a median follow-up of 73 months (range 0–122). Those with abnormal cytology were managed in accordance with standard practice guidelines.

We focused on the 2941 of 20 810 cohort women who tested positive for HPV by Hybrid Capture 2 and had satisfactory baseline Pap smears. We excluded 51 women because they had cytological evidence of CIN2+ at baseline. Of 2941 women who were positive for HPV, 88 (3%) were later diagnosed with histologically confirmed CIN3+, a much higher proportion than in women who tested negative for HPV (not included in this report).

We stratified viral loads of women positive for HPV into four groups on a log scale (1 to <10, 10 to <100, 100 to <1000, and ⩾1000 RLU/PC) and calculated absolute and relative risks for CIN3+ with 95% CIs. Compared with women who had viral loads of 1 to less than 10, the relative risk for CIN3+ in the first 9 months increased with increased load (table). However, no such trends were evident in later time intervals (table). For the entire follow-up of 122 months, relative risks did not increase at higher viral loads (table). Risk of CIN 3+ showed no significant trend by viral load (p=0·25). Additional analyses suggested that women with infections near the threshold of detectability (1–2 RLU/PC) had a slightly lower risk than women at all higher levels, possibly because of raised false-positivity including

Time after baseline smear	Viral load by HC2 (RLU/PC)	Women seen	Women with CIN3+	Absolute risk	Relative risk (95% CI)
0 to <9 months	1 to <10	1612	11	0·7	1·0 (reference group)
	10 to <100	756	10	1·3	1·9 (0·8–4·5)
	100 to <1000	455	7	1·5	2·3 (0·9–5·8)
	⩾1000	85	2	2·4	3·4 (0·8–15·3)
9 to <69 months	1 to <10	1235	23	1·9	1·0 (reference group)
	10 to <100	557	18	3·2	1·7 (0·9–3·2)
	100 to <1000	332	6	1·8	1·0 (0·4–2·4)
	⩾1000	58	0	0	0
69 to <122 months	1 to <10	697	6	0·9	1·0 (reference group)
	10 to <100	288	3	1·0	1·2 (0·3–4·8)
	100 to <1000	178	2	1·1	1·3 (0·3–6·4)
	⩾1000	32	0	0	0
Overall	1 to <10	1633	40	2·4	1·0 (reference group)
	10 to <100	765	31	4·1	1·7 (1·0–2·6)
	100 to <1000	457	15	3·3	1·3 (0·7–2·4)
	⩾1000	86	2	2·3	0·9 (0·2–3·9)

HC2=Hybrid Capture 2.

Relation between human papillomavirus viral load and development of CIN3+ during follow-up

cross-reactivity with non-carcinogenic types of HPV. Also, results from our ongoing work quantifying viral load using TaqMan PCR (Applied Biosystems, Foster City, CA, USA) suggest that some HPV infections with very low viral loads are missed by Hybrid Capture 2 and these women have an extremely low risk of subsequent CIN 3+.

Enrolment age (<30 or ⩾30 years) did not change the results. The trend of increased risk of CIN3+ with increased viral load seen in the earliest follow-up period was eliminated by adjustment for baseline cytological measurement (negative *vs* mild/equivocal CIN). Specifically, most women with higher viral loads had cytological abnormalities at baseline, and in these women, increasing HPV load had no relation to risk.

By contrast with the case-control study,[1] we conclude that HPV viral loads measured by Hybrid Capture 2 in broad categories was not associated with risk of CIN3+ over 10 years of follow-up in women with carcinogenic types of HPV. We have several possible explanations for the discordant findings. First, comparison of HPV viral load values between studies is difficult because data are not given in terms of an absolute measure of viral genomes. Second, our study looked at an additive measurement of viral load for 13 carcinogenic HPV types, whereas previous studies focused only on type 16. Third, our results might differ from those of other studies because of differences in sample collection. Our use of lavages (instead of spatulas that were probably used in the other study) might have led to an under-representation of cells infected with HPV from the transformation zone in which most CIN3+ develops.

Finally, cytological interpretations might have differed. Women with cytological abnormalities have higher viral loads and are at higher risk of progression to CIN3+ than women who have normal cytological samples.[3,4] However, investigators in the Scandinavian case-control study used Pap smears in a less sensitive but more specific interpretative pattern, as is common practice in Scandinavian countries compared with the USA.[5] Thus, some women with positive HPV of low-grade or equivocal lesions may have been judged as cytologically negative and not referred for colposcopy, permitting progression to CIN3. Accordingly, we postulate that increased risk of carcinoma in situ associated with higher HPV 16 viral load in women interpreted as cytologically normal in Scandinavia might have been weaker if USA cytological interpretations had been used. Our data suggest confounding between cytological abnormalities and HPV load, in that adjustment for baseline cytological interpretation eliminated the unadjusted trend in risk with increasing viral load seen early in follow-up. The relation between HPV viral load, concurrent cytological diagnosis, and risk of subsequent cervical carcinogenesis is still unclear.

Contributors

A Lorincz did the laboratory testing of clinical specimens, interpretation of data, and drafting of the report. P Castle interpreted the data, did the statistical analysis, and drafted the report. S Wacholder, P Gravitt, and J Schussler interpreted data and did statistical analyses. M Sherman and D Scott were the expert reviewers of the histologies and established final case status. They also reviewed and edited the report. B Rush and A Glass were responsible for coordinating field activities at Portland Kaiser and for data collection. M Schiffman was responsible for the idea of the study, statistical analysis, interpretation of data, and revising of the report. All these authors participated in the decision to submit this letter for publication.

Conflict of interest statement

A Lorincz is an employee of Digene Corporation.

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Postexposure prophylaxis against prion disease with a stimulator of innate immunity

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The absence of an immune response to prions—the infectious agents of scrapie, bovine spongiform encephalopathy (BSE), and Creutzfeldt-Jakob disease—might be related to the fact that these agents do not contain nucleic acids. We aimed to use CpG oligodeoxynucleotides, which have been shown to stimulate innate immunity, as a form of postexposure prophylaxis in mice. We inoculated healthy mice with brain homogenates from mice infected with the RML scrapie prion, and then injected CpG oligodeoxynucleotides. This postexposure prophylaxis with CpG oligodeoxynucleotides resulted in 38% longer survival times than treatment with saline (p<0·0001), or even longer after repeated application. The explanation for this finding remains to be elucidated, but the most likely is stimulation of TLR9-expressing cells of the innate immune system such as macrophages, monocytes, and dendritic cells. CpG oligodeoxynucleotides have not been shown to have adverse effects to human health and could therefore be considered as a therapeutic choice in postexposure prophylaxis.

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Development of postexposure prophylaxis for acquired human prion diseases—eg, variant and iatrogenic Creutzfeldt-Jakob disease (vCJD and iCJD)—is of high priority. Medical workers such as neurosurgeons, pathologists, nurses, morticians, histology technicians, and laboratory workers are at special risk of accidentally contracting iCJD. Accidental transmission to patients through certain surgical procedures has been noted.

The immune response to bacteria and viruses is triggered by, among other things, the presence of foreign nucleic acids.[1]